SOME EFFECTS OF HYPOPHYSECTOMY ON THE DOMESTIC HEN (*GALLUS DOMESTICUS*)

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This study is an attempt to explain some of the effects of hypophysectomy on the domestic hen, with the hope that it will contribute to the general knowledge of hormone deficiency in birds. The effects produced by the removal of the anterior pituitary gland from common laboratory mammals and amphibians are well understood. This is not the case, however, with birds. Consequently, satisfactory phylogenetic comparison between the amphibians, birds and mammamls is not possible. There is almost complete agreement that the deficiency of anterior pituitary hormones in birds causes regressive changes of secondary sex characteristics and of certain endocrine glands, notably the gonads, thyroids and adrenals.

MATERIALS AND METHODS

The animals used in this study, White Leghorn pullets, were hatched from the same brood in December, 1951 and acquired from the Ohio State University poultry farm in June, 1952. The selection of this animal is recommended because of its extensive use in experimental work, its ease of maintainence and handling and its high resistance to bacterial infection when surgically treated.

From a lot of twenty-four pullets three groups of five each were randomly selected and designated as: Group I, normal controls; Group II, hypophysectomized; and Group III, operated controls. The normal control animals were subjected to no experimental treatment, but were maintained under the same environmental conditions as the other animals. The anterior pituitaries of the hypophysectomized pullets were removed by the method described by Rothchild (1948). The operated control birds were subjected to the same treatment as the hypophysectomized pullets except that when the pituitary glands were exposed no attempt was made to remove them. Since Nalbandov and Card (1934) found that the hypoglycemia can be minimized in hypophysectomized hens by reducing the intervals between feedings, an additional hour of light was provided from 1:00 A.M. to 2:00 A.M.

Immediately after the death of a pullet the body was opened mid ventrally and the thyroids and ovaries were removed. These organs were weighed immediately and then fixed in Bouin's. The viscera were inspected for evidence of disease; there was no evidence in any of the pullets used in this study.

In order to determine the completeness of the operation the areas of the pituitaries in the sella turcica were dissected out, fixed in Bouin's, decalcified in dilute nitric acid and imbedded and sectioned in paraffin, as were the thyroids and ovaries. The ten micron sections were stained in azocarmine G-dioxane and counter-stained in orange G-aniline blue. Details are given by Guyer (1936).

RESULTS

Differences in behavior and appearance between the normal control and operated control birds were not observed after the latter had recovered from the effects of the surgical treatment. For several hours following the sham hypophysectomies these birds stood quietly and made little attempt to eat or drink. By the next day, however, these birds were alert and had regained a typical posture with head and tail feathers erect.

With the advent of sexual maturity the combs and wattles of these pullets increased in size and assumed a red, fleshy appearance. They laid eggs in regular cycles which varied with the individuals.

The weights of the thyroids of the two control groups compared closely and statistical analysis indicates that there is no significant difference between the means of these groups. The size and weight of each member of a pair of thyroids usually varied slightly. Microscopical examination did not indicate any notable

TABLE 1

Weight of thyroid glands in grams.

Group I Normal Control		Group II Hypophysectomized		Group III Operated Control	
1	0.300	6	0.164	11	0.296
2	0.318	7	0.170	12	0.364
3	0.280	8	0.050	13	0.296
4	0.248	9	0.146	14	0.242
5	0.232	10	0.318	15	0.322
means	0.276		0.169		0.307

Analysis of Variance

Source	df	mean squares	F
Between means.....	2	0.339	37.70
Within means.....	12	0.009	

Minimal significant difference between means 0.130

difference in the structural and apparent functional state of the glands from the same individual. No marked differences were observed in the thyroids from all the control animals (table 1).

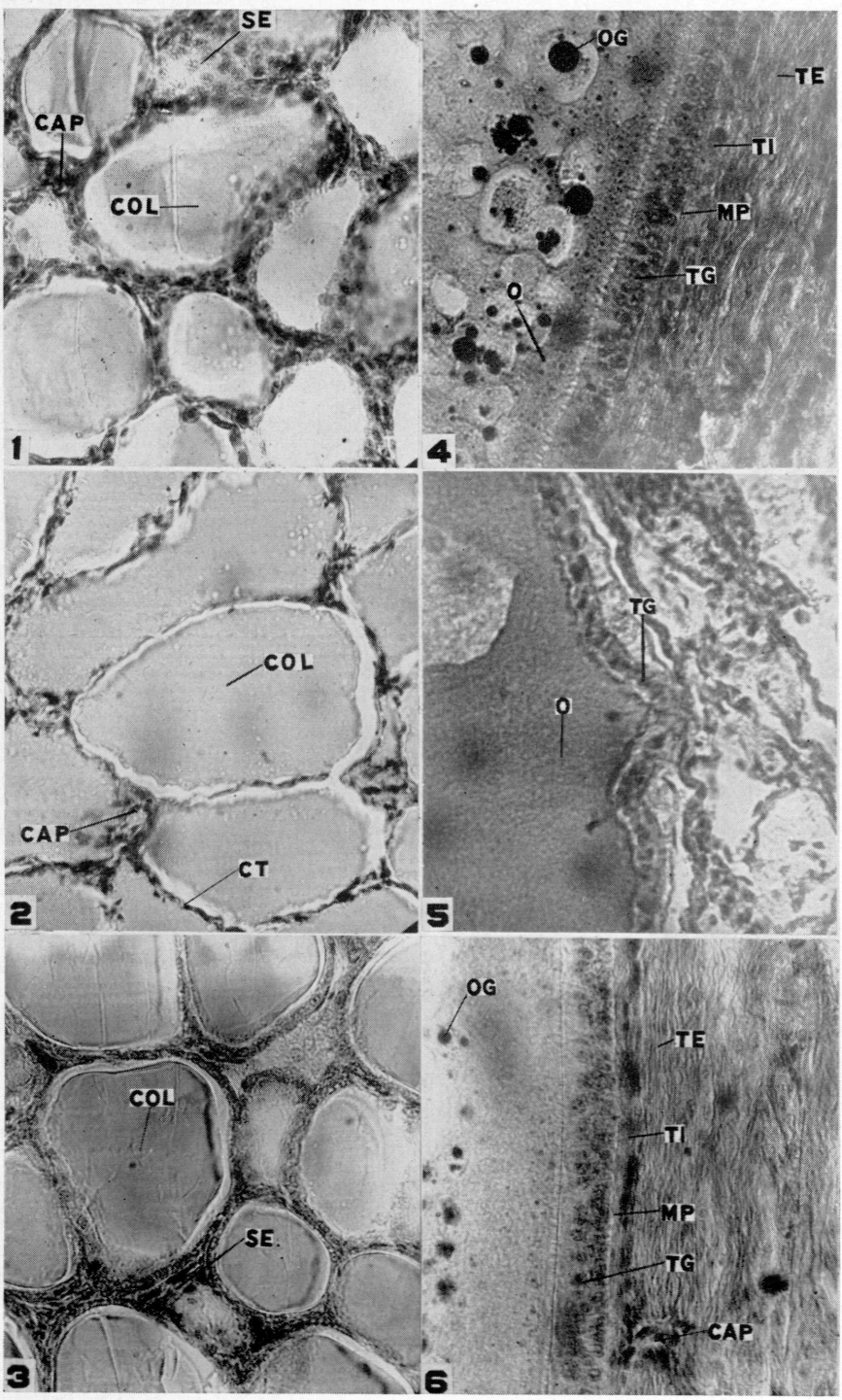
The comparative size of the vesicles in the glands was estimated by counting the number in the microscopic field under 660 × magnifications. Counts were made from several sections from each member of a pair of glands. The average number of vesicles for the operated controls and the normal controls was sixteen. The condition of the secretory epithelium of the vesicles of normal control and operated control thyroids was subjectively compared and did not appear to differ. Figures 1 and 3 show the structure of these glands. The thickness or height of

EXPLANATION OF FIGURES

1. Section through the thyroid gland of a normal control showing the condition of the secretory epithelium and colloids.
2. Section through the thyroid of an hypophysectomized pullet showing the loss of secretory epithelium and increased size of vesicles. There has been shrinkage of the colloid margins.
3. Section through the thyroid of an operated control pullet showing the condition of the secretory epithelium and colloids.
4. Section through an ovarian follicle of a normal control pullet showing the detailed structure of the öocyte and the thecal layers.
5. Section through the ovarian follicle of an hypophysectomized pullet showing the lack of integrated structure of the öocyte and thecal layers.
6. Section through an ovarian follicle of an operated control pullet showing the detailed structure of the öocyte and the thecal layers.

ABBREVIATIONS USED IN FIGURES

CAP.	Capillary	OG.	Oil globule
COL.	Colloid of thyroid gland	SE.	Secretory epithelium
CT.	Connective tissue	TE.	Theca externica
MP.	Membrana propria	TG.	Theca granulosa
O.	Oocyte	TI.	Theca internica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the epithelium was considered to be medium on the basis of descriptions by Hopkins (1935) and Turner (1948). In tangential sections of vesicles secretory cells were numerous. Although an extensive vascular network was present throughout the glands, the number of blood cells in the capillaries surrounding the follicles was not great.

Upon postmortem inspection of the two control groups the ovaries were large, irregular masses of soft, pink tissue which contained many ova of sizes from a few millimeters in diameter to 25 mm. The larger ova were bright yellow in contrast to the smaller, pale yellow ova which were still imbedded in the cortex of the ovary. The wet weight of the ovaries are given in table 2.

When sections from these ovaries were examined no differences in structure of the tissues of the two control groups were observed. As a criterion for comparison the condition of the follicular tissue surrounding the öocytes was studied. A ring of follicular cells, the theca granulosa, was observed in contact with the developing öocyte. This ring is a single layer of cuboidal cells which are active in the forma-

TABLE 2

Weight of ovaries in grams

Group I Normal Control		Group II Hypophysectomized		Group III Operated Control	
1	19.17	6	1.75	11	18.70
2	45.65	7	1.70	12	15.65
3	20.10	8	0.80	13	28.48
4	45.45	9	1.58	14	4.42
5	23.48	10	7.40	15	22.63
means	30.77		2.65		17.97

Analysis of Variance

Source	df	mean squares	F
Between means.....	2	991.37	10.93
Within means.....	12	90.72	

Minimal significant difference between means 13.13

tion of deutoplasm within the öocyte. The peripheral surface of these cells is smooth and is covered by a basement membrance, the membrana propria, which has a selective function in the supply of nutritive materials to the öocytes (Romanoff and Romanoff, 1949). Two reticular layers invest the structures described above. These are the theca internica and the theca externica. The theca internica is a highly vascular layer about three cells thick. The theca externica is composed of concentric layers of spindle-shaped cells and thick fibers. These relationships are shown in figures 4 and 6.

The appearance of the pituitaryless pullets immediately after the operation was similar to that described above for the operated controls. However, they did not recover as rapidly nor as completely in the next few days. In the first postoperative week the changes in the behavior and the appearance of these birds were not pronounced, but they were slightly less active and appeared to consume less food. No ovulations occured in any of the experimental birds after the anterior pituitary had been removed.

By the end of the second postoperative week the feathers of the hypophysectomized pullets began to take on an ivory cast and no longer lay smoothly along the body. The combs and wattles of these birds also regressed to low, pale ridges of hard tissue. There was some evidence of moulting, but this was not great.

As the length of time increased the effects ascribed to anterior pituitary deficiency became more obvious. Activity was rarely observed and water and food

consumption was much less. In the week preceeding the death of two of the pituitaryless pullets extremely moribund characteristics were observed. The birds stood quietly in a semi-squatting position with the neck flexed and the head slightly drooping. The eyes were usually closed and the tail feathers dragged on the bottom of the cage. One of these died in slight convulsion. Of the three remaining experimental birds, two died later without signs of the extreme conditions described above. The average length of postoperative life for these four birds was 46 days. The fifth experimental pullet exhibited many of the previously discussed changes for part of its postoperative life, but the character of these changes reversed after about five weeks. Gradual increases in activity, food consumption and comb and wattle size over a period of two weeks indicated that ablation of its pituitary had not been complete. Later evidence showed this assumption to be correct. This bird was killed along with those of the two control groups after the fourth experimental bird had died.

The thyroid glands of the hypophysectomized pullets were smaller than those of the controls and more difficult to locate. As seen in table 1, the weights of these thyroids were considerably less than those of either control group. This difference is supported by statistical analysis.

Microscopic examination of these thyroids showed a uniformly different condition from that observed in the normal thyroid. The most striking difference was the nearly complete disappearance of follicular epithelium. Large areas of the sections were studied before observing follicles which were lined with a low layer of these cells. Their nuclei were smaller than those seen in the secretory cells of the normal thyroids. In tangential cuts through the vesicles of these glands secretory cells were not seen. Counts were made of the number of vesicles seen in the field under 660 × magnifications and averaged ten. This index of size was used to compare with normal thyroids and showed that in addition to loss of epithelial tissue the colloids of these thyroids were larger, since fewer of them were seen in an equal area. The interstitial tissue which separates the follicles was greatly reduced and the capillaries imbedded in this tissue were more conspicious. The number of corpuscles in them appeared to be much greater than in normal thyroids. These relationships are shown in figures 1, 2 and 3.

The ovaries of the hypophysectomized birds were greatly reduced in size and contained no ova larger than 3 mm. These were imbedded in the dark red cortex of the ovary. Statistical analysis indicates that the mean weights of these ovaries differ significantly from the mean ovarian weights of the controls (table 2).

Microscopic observation showed that the follicles of these ovaries were not organized into the distinct regions as were the ovaries of the control animals. The theca granulosa was much lower and the nuclei were smaller. This ring of cells still adhered to the öocyte, but in most cases it and the basement membrane had broken away from the theca internica. This latter structure was not clearly distinguishable, but there seemed to be more blood cells in the capillaries of this area of the ovary of hypophysectomized birds than in the corresponding area of the ovary of control birds. There was little indication of concentric layers in the theca externica. The spindle-shaped cells were nearly absent and the recticular nature observed in the control ovaries was not observed. Within the öocyte there was no evidence of oil globules and the yolk platlets, which were seen in a few cases, were irregular masses. These conditions are shown in figures 4, 5 and 6.

No anterior pituitary tissue was seen in the sections from the four experimental birds which died. As was anticipated the anterior pituitary of bird number 10 was not completely excised; about two-thirds of this gland remained.

DISCUSSION

Not all of the changes which result from deficiency of anterior pituitary hormones in the domestic hen are understood, but the results of this study and

studies of others indicate that many of them can be attributed to the elimination or drastic reduction of functions which are regulated directly by these hormones or indirectly by their influence on other endocrine secretions.

The decrease in the size of the thyroids of hypophysectomized pullets and the loss or flattening of the secretory epithelium of their follicles was assumed to indicate that secretion of thyroid hormone from them was greatly reduced or lacking. Hartman (1946) says that the relative sizes of endocrine glands are an indication of their ability to produce hormones. Turner (1948) cites work which shows that the thickness of the secretory epithelium is indicative of the activity of the thyroid gland. Further, an indication of decreased thyroid secretion is the enlargement of colloids in conjunction with the reduction of epithelium. According to Hopkins (1935) the ratio of the volume of the colloid to the secretory epithelium increases as the secretory activity of the gland decreases. The principle function of thyroxin in warm-blooded animals is the regulation of metabolism. Since metabolism decreases by 25 to 30 per cent in most species when thyroid hormone is deficient (Fulton 1950) it can be assumed that the lethargy, lack of appetite and drooping posture of the experimental animals were, in part at least, due to this cause.

The great reduction in the size and weight of the ovaries of the birds after the removal of the anterior pituitaries indicates that normal function of these organs does not occur under conditions of deficiency of anterior pituitary hormones. Since all the experimental pullets were in a laying condition prior to the operation their ovaries must have been similar to those found in the normal birds. The lack of any mature ova in these ovaries indicates that the deutoplasm which had formerly accumulated within them had been resorbed. This observation is consistant with the reports of Hill et al., (1934) and Nalbandov and Card (1943). The microscopic structure of the normal ovarian follicle represents integrated functions of cells of various tissues which produce mature ova. The loss of this integration from follicles which are not influenced by anterior pituitary hormones indicates a regulation in which the hormones are involved. The specific nature of this regulation has not been demonstrated, but Fraps et al., (1942) were able to accelerate the formation and ovulation of mature ova in White Leghorn hens by intraperitoneal injections of follicle-stimulating hormone (FSH) and lutenizing hormone (LH), gonadotropic hormones of the anterior pituitary gland. That other factors are involved in ovulation was shown by Pearl and Surface (1916) when they failed to induce or augment ovulation in hens in which there was complete ovarian rest when they injected anterior pituitary extracts. The need for investigation of the hormonal regulation of ovarian function in the bird is apparent. Although a few studies report the effects of hormone deficiency on the avian ovary none was found which describes the microscopic structure of the ovarian tissue under these conditions.

The changes observed in the combs and wattles are attributed to the deficiency of ovarian hormones since investigators, who removed the ovaries, produced the same effects. The influence of ovarian hormones on secondary sex characteristics is also indicated by the rapid development of these structures at puberty. Latimer (1925) showed that the ovarian hormone estrogen is necessary for the maintainance of the comb and wattles.

The cause of death following hypophysectomy is not known; however, severe reduction of the level of blood glucose has been suggested by several investigators. Nalbandov and Card (1943) and Hill et al., (1934) showed that this low level of blood glucose in itself is not sufficient to cause death. They agree that death is due to drastic interference with metabloic processes, only one factor of which is the reduction of glucose in the circulation. Hill et al., (1934) suggest that the formation of glucose from endogenous protein does not take place in absence of estrogen. This factor was circumvented by preventing long intervals between

periods of eating in the hypophysectomized pullets used in this study as is suggested by Nalbandov and Card (1943). In a recent private communication Nalbandov expressed the opinion that if all extreme and rapid changes in the environment of an hypophysectomized hen are prevented, the animal should live out its normal life expectancy.

SUMMARY AND CONCLUSIONS

1. The removal of the anterior pituitary gland from White Leghorn pullets caused the thyroids and ovary to atrophy. Changes in behavior and external morphology resulting from this condition were lethargy, ruffled feathered coat, immediate cessation of ovulation and atrophy of the comb and wattles.

2. Changes in the structure of the thyroids are loss or reduction of the secretory epithelium, increased size of the colloids and loss of interstitial epithelium.

3. Changes in the structure of the ovary are resorption of deutoplasm from the developing ova and breakdown of thecal layers which surround the ova.

4. This study and other investigations indicate that a severe metabolic disturbance involving many factors is involved in the explanation of death following anterior pituitary ablation.

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